Photometric immersion refractometry: a method for determining the refractive index of marine microbial particles from beam attenuation

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Photometric immersion refractometry is a technique for determining the refractive index of particulate material. In this technique, the attenuation of light by a suspension of particles is measured as a function of the refractive index of the immersion medium. A minimum attenuation occurs at the refractive index of the medium equal to that of the particles. This technique can serve as a benchmark method for the refractive index determination because it is independent of assumptions invoked by other techniques, such as those based on the inversion of the spectral attenuation data. We present a rigorous model of the photometric immersion refractometry based on the anomalous diffraction approximation for the attenuation efficiency of particles. This model permits one to determine the average value of the real part of the refractive index of the particles, its variance, and the average imaginary part of the refractive index of the particles. In addition, the fourth moment of the particle size distribution can be determined if the concentration and shape of the particles are known. We analyze the sensitivity of this model to experimental errors and discuss the applicability of photometric immersion refractometry to marine microbial particles. We also present experimental results of this technique as applied to heterotrophic marine bacteria. The results indicate that the refractive index of these bacteria was narrowly distributed about the average value of 1.3886. © 1997 Optical Society of America

1. Introduction

The refractive index is an important characteristic of small particles in suspensions and aerosols. This importance stems essentially from two sources. First, the refractive index of a particle is related to, and thus can be used to infer, the composition of the particle. This relationship is reflected in a close correlation of the refractive index of minerals with their density.[1] Similar correlation has been established between the refractive index of biological cells and their content.[2,3] It was shown that the refractive index of biological cells is a measure of the intracellular solids concentration[4] and, in particular, of the intracellular carbon concentration.[5,6] In the case of biological cells, the refractive index is also affected by, and thus can be used to monitor, the physiological state of the cells.[6,7]

Second, the refractive index is an essential parameter in the modeling of light scattering and absorption by particles. In complex suspensions, such as seawater, better knowledge of the refractive indices of various particle classes is required to assess accurately the contributions of these classes to light scattering. For example, it has been postulated[8,9] that bacteria may contribute significantly to the optical properties of seawater, especially to the fraction of light backscattering associated with living microorganisms. The backscattering coefficient of seawater is an important parameter to the remote sensing of the marine environment and to the optimization of underwater imaging systems.

Among several methods of determining the refractive index of particles, a technique of immersion refractometry has gained an important place in biology and mineralogy. In the original version of that technique, as applied in biology, cells are suspended in an isotonic protein solution whose refractive index is adjusted to match that of the cells.[4] That technique requires tedious microscopic observations that were replaced, in photometric immersion refractometry,

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with measurements of the optical density (OD) of a suspension.[10–14] In photometric immersion refractometry, the OD of a suspension of cells is measured as a function of the refractive index of the immersion medium. When the latter index matches that of the cells, the minimum OD is observed. In few published investigations, the OD measurements were made with use of general purpose spectrophotometers that are not optimized for use with suspensions. Consequently, it is difficult to interpret the OD in terms of relevant properties of particles in the suspension, even though a simple model of the photometric immersion refractometry has been proposed.[13]

In this paper we derive generalized relationships between the beam attenuation of light by particles in a suspension and the refractive index of the immersion medium. These relationships are based on the anomalous diffraction approximation[15] for the attenuation efficiency of particles. We account for the polydispersity of the size and refractive index of the particles and also for the absorption of light by the particles. We also describe the results of a photometric immersion refractometry experiment and associated determinations of the refractive index of heterotrophic marine bacteria.

2. Significant Notations and Abbreviations

a_2, a_1, a_0 — Coefficients of a second-order polynomial used to approximate the OD of a suspension as a function of the refractive index of the immersion medium [Eq. (22)].

A (cm^{-1}) — A parameter expressing the ratio of the thickness of a suspension layer to the square of the wavelength of light in vacuum [Eq. (15)].

c (cm^{-1}) — Attenuation coefficient of a medium [Eq. (6)].

C_{at} (cm^2) — Attenuation cross section of a particle [Eq. (7)].

C_s (nondimensional) — Particle shape coefficient [Eq. (13)].

D (cm) — Diameter of a particle—a characteristic particle size [Eq. (3)].

$\langle D^3 \rangle$ (cm^3) — The third moment of the particle size distribution [Eq. (29)].

$\langle D^4 \rangle$ (cm^4) — The fourth moment of the particle size distribution [Eq. (27)].

D_{peak} (cm) — Peak diameter of the log-normal distribution [Eq. (B1)].

$F_{DM}(D, m)$ (cm^{-4}) — Generalized size refractive-index distribution function

FD_{max} (cm^{-4}) — A scale constant of the log-normal size distribution [Eq. (B1)].

$g(D)$ (cm^{-4}) — Frequency size distribution of particles [Eq. (4)].

G — A factor in the expression for $\langle k \rangle$ [Eq. (31)].

k (nondimensional) — Imaginary part of the refractive index of a particle relative to vacuum [Eq. (11)].

$\langle k \rangle$ (nondimensional) — Average k in a population of particles [Eq. (28)].

L (cm) — Thickness of a layer of suspension.

m (nondimensional) — Complex refractive index of a particle relative to vacuum [Eq. (11)].

n (nondimensional) — Real part of the refractive index of a particle relative to vacuum [Eq. (11)].

$\langle n \rangle$ — The average refractive index of a particle population [Eq. (26)].

n_s (nondimensional) — Refractive index of the medium surrounding a particle (immersion medium).

N (cm^{-3}) — Number concentration of particles with diameters D (cm) between D_{min} and D_{max}, and with refractive indices between m_{min} and m_{max} [Eq. (2)].

OD (nondimensional) — Optical density of a medium per centimeter of the medium thickness [Eq. (1)].

P (cm^2) — Geometric cross section of the particle projected onto a plane perpendicular to the direction of the propagation of light.

P_{avg} (cm^2) — Orientation-averaged geometric cross section of the particle projected onto a plane perpendicular to the direction of the propagation of light [for cylinders with hemispherical cups, see Eq. (44)].

Q_{at} (nondimensional) — Attenuation efficiency of a particle [Eqs. (8), (12), and (13)].

s (nondimensional) — Slope of a power-law approximation to the OD wavelength spectrum [Eq. (45)].

λ (cm) — Wavelength of light in vacuum.

ρ (nondimensional) — Dimensionless size of a particle [Eq. (9)].

σ_D (nondimensional) — Width parameter of the log-normal size distribution [Eq. (B1)].

σ_n^2 (nondimensional) — Variance of the refractive index in a particle population [Eq. (37)].

ADA — Anomalous diffraction approximation.

BSA — Bovine serum albumin.

LSQ — Least-squares approximation.

3. Theoretical Model of Photometric Immersion Refractometry

A. Single-Wavelength Model

In this section we develop a simple model relating the optical density (at a single wavelength) of a suspension of particles to the refractive index of the immersion medium. The major observables of our model are the optical density OD of the suspension and the real part n_s of the refractive index of the immersion

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medium. The OD can be expressed as follows:

$$\mathrm{OD} = cL \log e, \tag{1}$$

where c is the beam attenuation coefficient (for example, Ref. 16), and L is the thickness of the relevant layer of the suspension. Because the OD is defined in our model in terms of the beam attenuation coefficient, the spectrophotometer used to measure the OD must have an appropriate optical path geometry.[14,17] Also, it is assumed that a double-beam spectrophotometer is used so as to cancel the effect of residual light attenuation by the immersion medium on the OD measurements.

We assume that each particle in a population is homogeneous but the population may be polydisperse in respect to the particle size and refractive index. No assumptions about the polydispersity of the particles are made. However, the accuracy of certain predictions of this model improves if the particles are weakly polydisperse. The population can be described with the generalized size refractive-index distribution function $F_{DM}(D, m)$, defined as follows:

$$N = \iint F_{DM}(D, m)\mathrm{d}D\,\mathrm{d}m, \tag{2}$$

where N is the number concentration of particles with diameters D between $D_{\min}$ and $D_{\max}$ and with refractive indices between $m_{\min}$ and $m_{\max}$. The latter four variables serve as the limits of the integrals. The diameter of a particle is a characteristic dimension of the particle. In the present context, this dimension is defined as follows:

$$D = [(4/\pi)P]^{1/2}, \tag{3}$$

where P is the geometric cross section of a particle projected onto a plane perpendicular to the direction of the propagation of light. We assume that the refractive index of particles and their size are not correlated. With this assumption, discussed further in Appendix A, the function F_{DM} can be expressed as follows:

$$F_{DM}(D, m) = g(D)h(m), \tag{4}$$

where

$$h(m) \equiv h(n) \equiv h(k). \tag{5}$$

Equation (5) applies, for example, when variations in the refractive index of marine microbial cells are caused by dilution of cell contents with water.[2]

Using the generalized distribution function of the particle population, we can express the beam attenuation coefficient of the population as follows:

$$c = \iint C_{\mathrm{at}}(D, m, \lambda)F_{DM}(D, m)\mathrm{d}D\,\mathrm{d}m, \tag{6}$$

where

$$C_{\mathrm{at}} = Q_{\mathrm{at}}(D, m, \lambda)P(D) \tag{7}$$

is the attenuation cross section and Q_{at} is the attenuation efficiency of a particle (for example, Ref. 16). The attenuation efficiency Q_{at} is a function of the refractive index of the particle m, the refractive index of the surrounding medium n_s, the particle diameter D, and the wavelength of light in vacuum λ. Equation 6 is valid under the assumption that single scattering dominates the light-scattering process in the suspension. Such a situation occurs if particles are far away from each other, that is, if the interparticle distance is much greater than λ. We discuss this further later in this section.

By using the anomalous diffraction approximation[15] (ADA), we have

$$\begin{aligned} Q_{\mathrm{at}} = {} & 2 - (4/\rho)\exp(-\rho \tan\beta)\cos\beta \sin(\rho - \beta) \\ & - (4/\rho^2)\exp(-\rho \tan\beta)\cos^2\beta \cos(\rho - 2\beta) \\ & + (4/\rho^2)\cos^2\beta \cos 2\beta, \end{aligned} \tag{8}$$

where

$$\rho = 2\pi(D/\lambda)(n - n_s), \tag{9}$$

$$\tan\beta = k/(n - n_s), \tag{10}$$

$$m = n - ik, \tag{11}$$

where n and k are, respectively, real and imaginary parts of the refractive index of the particle, n_s is the refractive index of the medium surrounding the particle, and $i = (-1)^{1/2}$. All indices are given relative to the vacuum.

The anomalous diffraction theory applies when the $|m - n_s| \ll n_s$ and when the particle is "large" compared with the wavelength of light in the medium surrounding the particle. We use quotation marks because the ADA works in a surprisingly wide particle size range.

Equation (8) can be well approximated[15] by the following formula when $\rho \approx 0$ (as a consequence of $n - n_s \approx 0$):

$$Q_{\mathrm{at}} \approx 2(\pi/\lambda)^2D^2[(n - n_s)^2 - k^2] + (8/3)(\pi/\lambda)Dk. \tag{12}$$

Equation (12) can be transformed to the following form:

$$\begin{aligned} Q_{\mathrm{at}} \approx {} & C_s4(\pi^2/\lambda^2)D^2[(n - n_s)^2 - k^2] + C_s(16/3) \\ & \times (\pi/\lambda)Dk, \end{aligned} \tag{13}$$

where $C_s = 1/2$ represents the particle shape factor for a sphere. We factored out 1/2 and replaced it by a more general coefficient C_s so as to account for the influence of the particle shape on Q_{at}. For example, for long, thin, and randomly oriented uniform cylinders $C_s = 4/3$, as can be inferred from Ref. 18 [substitute for the first Struve function in Eq. (51) of Ref. 18 its small-argument approximation $2\rho^2/(3\pi \sin^2\beta)$, where β is the angle between the direction of the incident radiation and the axis of the cylinder]. In this latter case, D represents the diameter of the cylinder.

From Eqs. (13), (7), (6), (4), and (1) we obtain the

following expression for the optical density of a suspension:

$$\mathrm{OD} \approx C_e A\left\{\int D^4 g(D)\mathrm{d}D \int [(n-n_s)^2 - k^2]h(m)\mathrm{d}m + (4/3)(\lambda/\pi)\int D^3 g(D)\mathrm{d}D \int kh(m)\mathrm{d}m\right\}, \quad (14)$$

where A is defined as follows:

$$A = \pi^3 \log e L \lambda^{-3}. \quad (15)$$

By using the definition of an rth moment, $\langle x^r\rangle$, of distribution f of variable x:

$$\langle x^r\rangle = \int x^r f(x)\mathrm{d}x \Big/ \int f(x)\mathrm{d}x \quad (16)$$

and making use of Eq. (5), we can transform Eq. (14) as follows:

$$\mathrm{OD} = C_e AN[\langle D^4\rangle\langle (n-n_s)^2\rangle - \langle D^4\rangle\langle k^2\rangle + (4/3)(\lambda/\pi)\langle D^3\rangle\langle k\rangle], \quad (17)$$

where N is the number concentration of particles [Eq. (2)]. Furthermore, because

$$\langle x^2\rangle = \sigma_x^2 + \langle x\rangle^2, \quad (18)$$

where σ_x^2 is the variance of variable x, we obtain

$$\mathrm{OD} \approx C_e AN\langle D^4\rangle n_s^2 - C_e AN\langle D^4\rangle 2\langle n\rangle n_s + C_e AN\langle D^4\rangle(\sigma_n^2 + \langle n\rangle^2) + C_e AN(4/3)(\lambda/\pi)\langle D^3\rangle\langle k\rangle, \quad (19)$$

where σ_n^2 is the variance of the real part of the refractive index of the particles. In deriving Eq. (19) we neglected a term involving $\langle k^2\rangle$ that, for realistic values of k, is much smaller than the term involving $\langle k\rangle$. From Eq. (19) it follows that σ_n^2 and $\langle k\rangle$ cannot be separated with single-wavelength measurements of OD versus n_s. We discuss below how these two parameters can be separated with multiwavelength immersion refractometry.

The minimum optical density occurs at $n_s = \langle n\rangle$ and is equal to

$$\mathrm{OD}_{\min} \approx C_e AN[\langle D^4\rangle\sigma_n^2 + (4/3)(\lambda/\pi)\langle D^3\rangle\langle k\rangle]. \quad (20)$$

If the effect of the absorption of light by the particles on the $\mathrm{OD}_{\min}$ is dominant, then the $\mathrm{OD}_{\min}$ can be expressed as follows:

$$\mathrm{OD}_{\min} \approx C_e AN(4/3)(\lambda/\pi)\langle D^3\rangle\langle k\rangle. \quad (21)$$

Equation (19) is an equation of a second-order polynomial in the refractive index of the medium n_s:

$$\mathrm{OD} = a_2 n_s^2 + a_1 n_s + a_0. \quad (22)$$

The coefficients of Eq. (22) can be obtained by one fitting this equation to results of measurements of the OD of a suspension versus the refractive index of the immersion medium. A comment about such measurements is appropriate. The domain of the photometric immersion refractometry is a small range of the refractive indices of the immersion medium about $n_s = \langle n\rangle$. This implies relatively low values of the OD and significant relative errors of the OD measurements. The importance of these errors can be reduced when the optical density is increased, i.e., by the use of a concentrated suspension. However, the particle concentration cannot be too high, otherwise multiple scattering of light in the suspension may prevent one from using the measured OD as an estimate of the single-scattering OD assumed in this model. From the discussion of multiple scattering (for example, Ref. 19) it follows that the OD of the suspension should be significantly less than approximately 0.4 (at a path length of 1 cm).

By comparing Eqs. (19) and (22) we can express coefficients a_2, a_1, and a_0 as functions of the characteristics of the particle population:

$$a_2 = C_e AN\langle D^4\rangle, \quad (23)$$

$$a_1 = -C_e AN\langle D^4\rangle 2\langle n\rangle, \quad (24)$$

$$a_0 = C_e AN[\langle D^4\rangle(\sigma_n^2 + \langle n\rangle^2) + (4/3)(\lambda/\pi)\langle D^3\rangle\langle k\rangle]. \quad (25)$$

Equations (23) and (24) can be solved for the average real part of the refractive index of the particles:

$$\langle n\rangle = -a_1/(2a_2). \quad (26)$$

The retrieval of $\langle n\rangle$ is not affected by the variability in the real part of the refractive index of the particles nor by the absorption of light by these particles.

If we know the number concentration of particles N and the particle shape coefficient C_e, we can also obtain an estimate of the fourth moment of the particle size distribution from the following equation, resulting from Eq. (23):

$$\langle D^4\rangle = a_2/(C_e AN). \quad (27)$$

Different functional forms of the size distribution can yield the same $\langle D^4\rangle$. Thus the form of the size distribution cannot be inferred from photometric immersion refractometry measurements without additional assumptions. The $\langle D^4\rangle$ is a biased estimate of $\langle D\rangle$ with the bias depending on the particle size distribution (see Appendix B).

If it is known *a priori* that the effect of absorption on the $\mathrm{OD}_{\min}$ is dominant, then $\langle k\rangle$ can be calculated from Eq. (21) as follows:

$$\langle k\rangle \approx \mathrm{OD}_{\min}/[C_e AN(4/3)(\lambda/\pi)\langle D^3\rangle]. \quad (28)$$

The right side of Eq. (28) involves the third moment of the size distribution $\langle D^3\rangle$, which cannot be extracted from the coefficients a_0, a_1, and a_2 of Eq. (22) without the knowledge of $\langle k\rangle$. However, for a log-normal size distribution [Eq. (B1)], which is an adequate approximation of the marine particle size

distribution (see Appendix B), it can be shown that

$$\langle D^3 \rangle = \langle D^4 \rangle^{3/4} \exp[-(3/2)\sigma_D^2], \tag{29}$$

where σ_D is the width parameter of the size distribution. By replacing OD_{min} with $a_0 - a_1^2/(4a_2)$, which defines the minimum of the polynomial in Eq. (22), and by using Eq. (29), we obtain

$$\langle k \rangle \approx G[a_0/a_2^{3/4} - a_1^2/(4a_2^{7/4})], \tag{30}$$

where factor G is defined as follows:

$$G = \{3\pi \exp[(3/2)\sigma_D^2]\}/[4\lambda(C_e AN)^{1/4}]. \tag{31}$$

Considering the variability of σ_D (Appendix B), the exponential term in most cases will assume a value between 1 to approximately 4.5, with the most probable value of approximately 1.5. Thus, without prior knowledge of the form of the size distribution, the average imaginary part of the refractive index of the particles can be determined only to a factor of 1 to approximately 4.5 of the actual value. The accuracy of this determination can be improved if the size distribution is known.

The standard deviations of the parameters $\langle n \rangle$, $\langle k \rangle$, and $\langle D^4 \rangle^{1/4}$ can be estimated from the following equations resulting from a linearized error propagation model,[20] including the covariances of the coefficients a_0, a_1, and a_2 of Eq. (22):

$$\sigma(\langle n \rangle) = \{[1/(2a_2)]^2 \sigma^2(a_1) + [a_1/(2a_2^2)]^2 \sigma^2(a_2) - [a_1/(2a_2^3)]\mathrm{cov}(a_1, a_2)\}^{1/2}, \tag{32}$$

$$\begin{aligned}\sigma(\langle k \rangle) = G\{&[1/(a_2^{3/4})]^2\sigma^2(a_0) + [a_1/(2a_2^{7/4})]^2\sigma^2(a_1) \\ &+ [-(3/4)a_0/a_2^{7/4} \\ &+ (7/16)a_1^2/a_2^{11/4}]^2\sigma^2(a_2) \\ &- (a_1/a_2^{10/4})\mathrm{cov}(a_0, a_1) + [-(3/2)a_0/a_2^{10/4} \\ &+ (7/8)a_1^2/a_2^{14/4}]\mathrm{cov}(a_0, a_2) \\ &+ [(3/4)a_0a_1/a_2^{14/4} \\ &+ (7/16)a_1^3/a_2^{18/4}]\mathrm{cov}(a_1, a_2)\}^{1/2},\end{aligned} \tag{33}$$

$$\sigma(\langle D^4 \rangle^{1/4}) = \{[(1/4)(AN)^{-1/4}a_2^{-3/4}]^2\sigma^2(a_2)\}^{1/2}. \tag{34}$$

We stress that coefficients a_0, a_1, and a_2 must be known with sufficient numerical accuracy for Eqs. (30), (32), and (33) to produce meaningful results, because the right sides of these equations are differences between nearly equal numbers. This is a result of high correlations, typically better than 0.999, between the coefficients a_0, a_1, and a_2. Such correlations are mathematical artifacts due to expressing the OD = const $(n - n_s)^2$ as a polynomial in Eq. (22). However, the need for a high numerical precision of a_0, a_1, and a_2 does not imply that the errors of these coefficients, as calculated with the least-squares (LSQ) polynomial fitting procedure, must be accordingly small for the retrieval of the particle characteristics. Spreadsheet software for planning and analyzing a single-wavelength photometric immersion refractometry experiment is available for a nominal fee from M. Jonasz.

B. Case of Negligible Absorption of Light by Particles

The imaginary part k of the refractive index of heterotrophic marine bacteria is estimated to be of the order of 0.0001 or less.[9] For a typical size distribution of these bacteria, the contribution of the term involving $\langle k \rangle$ to the OD_{min} [Eq. (20)] is generally below the OD measurement error. Such particles are considered to exhibit negligible absorption of light in the context of this model. A minimum k of pigmented phytoplankton cells in the visible spectral region, which is located typically in the green, is of the order of 0.001 (Ref. 21). In such a case, the contribution of the $\langle k \rangle$ term to the OD_{min} can be comparable or greater than the OD measurement error. Hence the case of negligible absorption of light would not apply to phytoplankton within the visible spectral region. However, the absorption of light in phytoplankton cells (and consequently also k) rapidly vanishes toward the long-wavelength end of the visible range and in the near infrared.[21] Thus the assumption of negligible assumption of light would apply to phytoplankton in this spectral region.

By setting $\langle k \rangle = 0$ in Eq. (20) we obtain

$$OD_{min} \approx C_e AN\langle D^4 \rangle \sigma_n^2. \tag{35}$$

Similarly, Eq. (25) reduces to

$$a_0 = C_e AN\langle D^4 \rangle(\sigma_n^2 + \langle n \rangle^2). \tag{36}$$

From Eqs. (23), (24), and (36) we find the variance of the refractive index of particles in the population:

$$\sigma_n^2 = a_0/a_2 - [a_1/(2a_2)]^2. \tag{37}$$

The standard deviation of that estimate can be expressed as follows with the linearized error propagation model[20]:

$$\begin{aligned}\sigma(\sigma_n^2) = \{&[1/(a_2)]^2\sigma^2(a_0) + [a_1/(2a_2^2)]^2\sigma^2(a_1) \\ &+ [-a_0/a_2^2 + a_1^2/(2a_2^3)]^2\sigma^2(a_2) \\ &- (a_1/a_2^3)\mathrm{cov}(a_0, a_1) + 2[-a_0/a_2^3 \\ &+ a_1^2/(2a_2^4)]\mathrm{cov}(a_0, a_2) - 2[-a_0a_1/(2a_2^4) \\ &+ a_1^3(4a_2^5)]\mathrm{cov}(a_1, a_2)\}^{1/2},\end{aligned} \tag{38}$$

$$\sigma(\sigma_n) = [1/(4\sigma_n^2)\sigma^2(\sigma_n^2)]^{1/2}. \tag{39}$$

We again stress that coefficients a_0, a_1, and a_2, for reasons discussed above, must be known with sufficient numerical accuracy for Eqs. (37) and (38) to produce meaningful results.

C. Multiwavelength Photometric Immersion Refractometry

Consider first the case of particles that do not absorb light, and assume for simplicity that the distribution of the refractive index of particles is narrow. Then

$\langle k \rangle = 0$, $\sigma_n^2 \approx 0$, and $\langle n \rangle \approx n$. From Eq. (19) we have

$$[n(\lambda_1) - n_s(\lambda_1)]/[n(\lambda_2) - n_s(\lambda_2)] = [\mathrm{OD}(\lambda_1)/\mathrm{OD}(\lambda_2)]^{1/2}(\lambda_1/\lambda_2). \quad (40)$$

The refractive index of the medium n_s can be obtained separately as a function of wavelength. Thus, from the OD spectrum for a refractive index of the immersion medium near that of the OD minimum and from the refractive index of particles at a single wavelength (for example, λ_1), we can determine the dispersion of the refractive index of the particles.

Multiwavelength measurements are necessary for the retrieval of $\langle k \rangle$ and σ_n^2 in the case of nonnegligible absorption of light by the particle. Variables σ_n^2 and $\langle k \rangle$ in Eq. (20) each have a unique wavelength spectrum. If these spectra are known, up to a constant factor each, the latter two variables can be expressed as follows:

$$\sigma_n^2 = K_n S_n^2(\lambda), \quad (41)$$

$$\langle k \rangle = K_k S_k(\lambda), \quad (42)$$

where factors K are independent of wavelength, and both $S_n(\lambda)$ (the spectrum of the real part of the refractive index of a particle) and $S_k(\lambda)$, are assumed to be the same for all particles in the suspension, as a consequence of Eq. (5). After substituting Eqs. (41) and (42) into Eq. (20) we obtain

$$\mathrm{OD}_{\min}(\lambda) \approx C_e A N[\langle D^4 \rangle K_n S_n^2(\lambda) + (4/3)(\lambda/\pi) \times \langle D^3 \rangle K_k S_k(\lambda)]. \quad (43)$$

We can now calculate the factors K by measuring the $\mathrm{OD}_{\min}$ at two different wavelengths and solving the resulting set of linear equations (one equation of type 43 for each wavelength). It is assumed here that the real part n of the refractive index of the particles at these two wavelengths is nearly the same. Such an assumption can be made because (1) variations of n are generally estimated at less than approximately 0.004 in the visible[21] and (2) the OD varies slowly with $n - n_s$ near the OD minimum.

4. Numerical Simulation

A simulation was carried out for particles that do not absorb light. The size distribution was assumed to be log normal [Eq. (B1)] with $D_{\mathrm{peak}} = 0.4\ \mu\mathrm{m}$, $FD_{\max} = 4 \times 10^8\ \mathrm{cm}^{-3}\ \mu\mathrm{m}^{-1}$, and $\sigma_D = 0.57$ (Fig. 1). This distribution yields the number concentration of the particles of $2.7 \times 10^8\ \mathrm{cm}^{-3}$. Each particle is homogeneous, but particles in different size classes can have different real refractive indices. The refractive index of particles in a size class was randomly sampled from a uniform probability distribution with a spread of ± 0.0025 around a central value of 1.39. The OD of the suspension was calculated $\lambda = 0.55\ \mu\mathrm{m}$ with the ADA for spheres in two cases: (1) the exact optical density (Fig. 2) and (2) the optical density from case 1 with superimposed 10% errors randomly sampled from a uniform probability distribution. The error of numerical integration was approximately 0.1% of the OD. As seen in Fig. 1, we include all significant particle size classes in the integration size range.

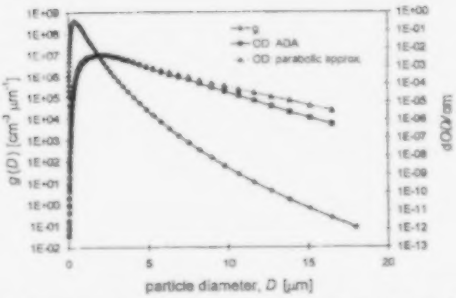

Fig. 1. Log-normal particle size distribution (left axis) used in the simulation of the photometric immersion refractometry (Table 1 and Fig. 2): $g(D) = FD_{\max} \exp[-(\ln D - \ln D_{\mathrm{peak}})^2/(2\sigma_D^2)]$, where $FD_{\max} = 4 \times 10^8\ \mathrm{cm}^{-3}\ \mu\mathrm{m}^{-1}$, $D_{\mathrm{peak}} = 0.4\ \mu\mathrm{m}$, and $\sigma_D = 0.57$. The number concentration of the particles is $2.7 \times 10^8\ \mathrm{cm}^{-3}$. The contributions of the various particle size classes to the total optical density (OD, right axis) were calculated with the anomalous diffraction approximation to $Q_{\mathrm{ext}}(n_s)$ for spheres at a wavelength of light of 0.55 μm in vacuum and at the refractive index of 1.36 of the immersion medium. The refractive index of particles in each size class was sampled from a uniform probability distribution with a mean of 1.39 and a spread of ± 0.0025. The contributions to the OD obtained with the parabolic approximation to $Q_{\mathrm{ext}}(n_s)$ are also shown (total OD error ≈ +5%).

To retrieve the characteristics of the particles, a parabola [Eq. (22)] was fitted to the OD versus n_s data with the LSQ algorithm. The coefficients of the parabola were then used to calculate $\langle n \rangle$, σ_n^2, and $\langle D^4 \rangle^{1/4}$ using Eqs. (26), (37), and (27). The results are summarized in Table 1.

In our simulation we aimed at reproducing the main features of our experiment: the magnitude of OD and the dependence of OD on the wavelength of light (Fig. 3). For this reason, we used a size distribution with $FD_{\max}$ and σ_D that are greater than those parameters of the experimental size distribution of

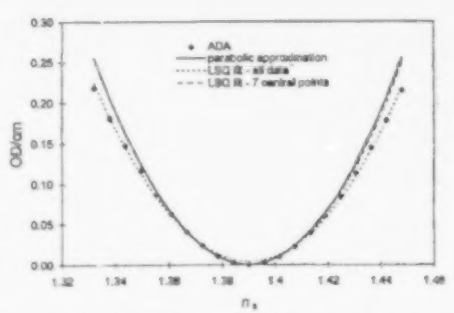

Fig. 2. Optical density OD of a particle suspension (size distribution in Fig. 1) as a function of the refractive index of the immersion n_s. The OD was calculated with the anomalous diffraction approximation (ADA) for spheres. By using the central 7 points ($|\langle n \rangle - n_s| < 0.02$) one obtains much better fit to the parabolic approximation to ADA than by using all data points ($|\langle n \rangle - n_s| < 0.06$).

Table 1. Summary of the Simulation of the Photometric Immersion Refractometry

Parameter Name	No Errors		10% Errors		Actual Values
	All Data	Central 7 Data	All Data	Central 7 Data	
$\langle n \rangle$	1.39026 ± 0.00016	1.39027 ± 1.1E-05	1.39048 ± 0.0002	1.39019 ± 4.6E-05	1.3902
σ_n^2	5.91E-05 ± 1.74E-05	1.9E-06 ± 3.9E-07	6.5E-05 ± 2.1E-05	3.1E-06 ± 1.6E-06	1.3E-06
$\langle D^4 \rangle^{1/4}$ (μm)	1.01977 ± 0.00262	1.05554 ± 0.00058	1.01854 ± 0.0032	1.04891 ± 0.00239	1.06

For the columns marked Central 7 Data, the data from within the range of $|\langle n \rangle - n_s| < 0.02$ were used. This reduces the error of approximation of the OD versus n_s with a second-degree polynomial in n_s. A single standard deviation is given following the ± sign.

single cells so as to account for the contribution to OD of cell aggregates in the experiment. By using the experimental size distribution of single bacterial cells we would have obtained OD that is less than that shown in Fig. 3 by more than an order of magnitude.

As can be seen from Figs. 1 and 2, and from Table 1, the accuracy of the parabolic approximation to the OD increases with decreasing $|\langle n \rangle - n_s|$. For the particle size distribution used, the parabolic approximation error decreases from approximately +20% at $|\langle n \rangle - n_s| = 0.058$ to approximately +5% at $|\langle n \rangle - n_s| = 0.02$. The accuracy of the retrieved particle characteristics changes accordingly (Table 1). An estimate of the average refractive index of the particles is least dependent on the refractive-index range used to obtain the LSQ estimates of coefficients a_0, a_1, and a_2. However, an estimate of the variance of the refractive index of the particles and an estimate of the fourth moment of the size distribution are affected significantly by the selection of that range.

5. Photometric Immersion Refractometry Experiment

A. Bacterial Culture

The free-living heterotrophic marine bacterial culture was grown in 70% diluted seawater enriched with nutrients. The term free-living is here an antonym of the term attached to other particles. The bacterial cells were isolated from seawater off Bermuda by Dr. Jed Fuhrman. We prepared the 70% seawater solution by diluting seawater, sampled near Catalina Island off Southern California, with deionized water and filtering this solution through a 0.22-mm Nuclepore filter. The filtrate was used to prepare the growth medium that contained 0.25 g of peptone and 0.25 g of casamino acid per 1 dm^3 of solution. The growth medium was autoclaved and filtered through 0.22-mm Nuclepore filters to remove precipitate formed during the autoclaving. Patches of bacterial colonies grown on solid agar were transferred to the growth medium and grown for 2 days at $T \approx 24$ °C with continuous stirring.

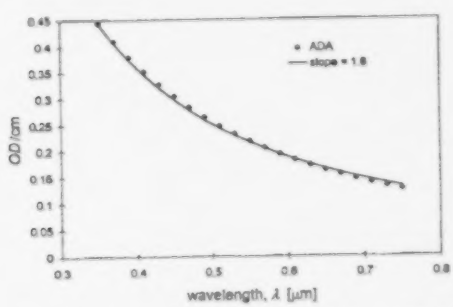

Fig. 3. Wavelength spectrum (solid squares) of the optical density OD of a suspension of particles (size distribution in Fig. 1) calculated with the anomalous diffraction approximation (ADA). The refractive index of the immersion medium equals 1.332. The power-law OD spectrum (const × λ^{-s}, line) is characteristic of our experiment with marine bacteria.

Bacterial suspension with a concentration of approximately 10^5 cells/cm^3 was filtered through 3-μm Nuclepore filters. The filtrate was centrifuged at 10 °C and an acceleration of approximately 8000 g for 15 min. The supernatant was discarded and the bacteria pellets were dispersed in approximately 20 cm^3 of filtered 70% seawater. When a standard, 1-cm quartz cuvette with the bacterial suspension was visually examined, the suspension appeared translucent (no image was transmitted through the cuvette). This stock bacterial suspension was diluted 16 times for the OD measurements. At $\lambda = 0.589$ μm, at which we made the measurements, the absorption of visible light by the bacteria was negligible.

The bacterial cells were somewhat larger and more elongated than typical marine bacteria that grow in natural environments where nutrients are less abundant. The cells had cylindrical shapes with a diameter-to-length ratio typically of approximately 1:5. The bacterial suspension also contained visually noticeable aggregates. The cells were stained with acridine orange and were filtered onto a black 0.22-mm Nuclepore filter for enumeration.[22] The enumeration sample was taken in the middle of the session of the OD measurements. Several photographs (color slides) of randomly selected areas on the filter were taken with an epifluorescent microscope at a magnification of 1000. The bacteria appear bright in the photographs (Figs. 4 and 5).

The size distribution of single cells, expressed as a function of the spherical equivalent diameter, is shown in Fig. 6. The size distribution was approximated by a log-normal function [Eq. (B1)] with $D_{peak} = 0.34$ μm, $FD_{max} = 1.85 \times 10^8$ cm^{-3} μm^{-1}, and $\sigma_D = 0.4$ for the number concentration of the cells of 1.3×10^8 cm^{-3}. We determined the size distribution by measuring the lengths and widths of over 400 images of single cells (including small aggregates of 5

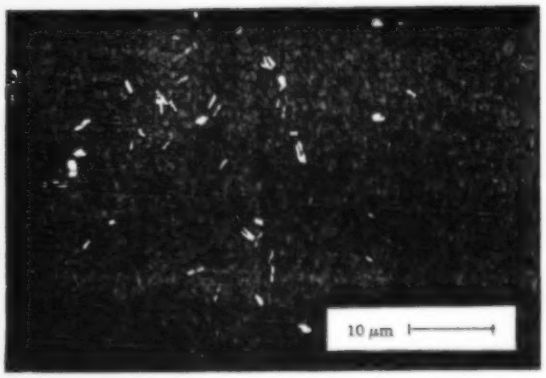

Fig. 4. Marine bacteria used in the immersion refractometry experiment. Bacteria were stained with acridine orange and viewed with an epifluorescent microscope.

to 8 cells) projected onto a screen from the color slides. Thus the size distribution does not account for the large aggregates, whose concentration was smaller than that of single cells by several orders of magnitude. The spherical equivalent diameter is defined here as a diameter of a sphere with the projected area (cross section) equal to the orientation average cross section of the cell. The orientation average cross section P_{avg} of a cell was approximated by that of a cylinder with hemispherical end cups and was calculated as follows:

$$P_{avg} = (1/4)\pi D_{cyl} L_{cell}, \tag{44}$$

where D_{cyl} is the diameter of the cylindrical part of the cell, and L_{cell} is the total length of the cell, including the hemispherical cups. The term (1/4) in Eq. (44) results from a theorem[15,33] stating that the orientation average cross section of a convex particle equals 1/4 of the particle's surface area.

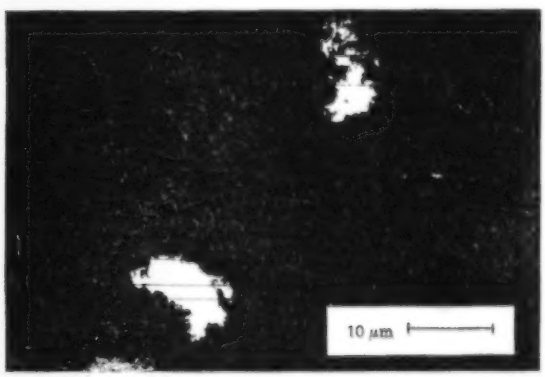

Fig. 5. Aggregates of marine bacteria from the immersion refractometry experiment. Bacteria were stained with acridine orange and viewed with an epifluorescent microscope.

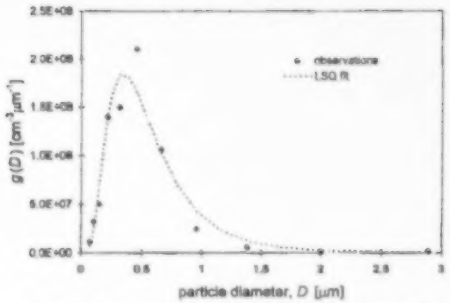

Fig. 6. Single-cell size distribution of marine bacteria in our experiment. The particle diameter is the diameter of a volume-equivalent sphere. A log-normal function $g(D) = FD_{max} \exp[-(\ln D - \ln(D_{peak}))^2/(2\sigma_D^2)]$ was fitted to the data with the least squares (LSQ). The fit parameters are $FD_{max} = 1.86 \times 10^8$ cm^{-3} μm^{-1}, $D_{peak} = 0.34$ μm, and $\sigma_D = 0.4$. The number concentration of cells is 1.3×10^8 cm^{-3}. We calculated the LSQ fit by letting the weight of a data point be equal to its y value, as follows from the Poisson probability distribution of the number concentration of bacteria.

B. Immersion Medium

We used an aqueous solution of bovine serum albumin (BSA) as an immersion medium. BSA solution is a nontoxic high-refractive index liquid suitable for use with micro-organisms. The molecular weight of BSA is sufficiently large to prevent the compound from penetrating the cellular wall of bacteria.[4,11,12,14] Therefore the refractive index obtained with such immersion medium relates to the whole cell. A 30% (wt./vol.) BSA solution (Sigma, Inc.) was concentrated to approximately 46% by slow evaporation at 40 °C under ambient pressure. Such concentrated BSA solution had a refractive index $n_D = 1.415$ (at $\lambda = 0.589$ μm; sodium-D line) and served as a stock solution of the immersion medium in our experiment.

C. Preparation of Samples and Reference Solutions

Using the stock solutions of BSA and deionized ultrafiltered water, we prepared a series of ten reference solutions with no bacteria. The volume of each reference consisted of a fixed amount of 70% seawater and a fixed amount of a BSA solution in deionized water. The partial volumes of BSA and deionized water in the BSA solution were calculated with the Gladstone–Dale mixing rule to yield a desired final refractive index of the entire reference. We prepared a corresponding series of ten samples by adding a volume of concentrated stock of bacteria equal to that of the fixed amount of 70% seawater in the reference solution. The lowest refractive index (1.332) in each series was nearly that of water. The highest refractive index (1.41) was nearly that of the 46% BSA stock solution.

For each sample, we added bacterial stock just before the measurements of the OD and refractive index because we were concerned that variations in the salinity of the solutions may affect the optical properties of the bacteria. Although the bacteria were

grown at a salinity of approximately 2.4%, the salinity of the samples ranged approximately from 0.85% (the highest refractive index solution) to 0.2% (the lowest refractive index solution). Therefore, prior to the experiment, we tested the effect of variations in the salinity of a suspension on the OD by transferring cells from the original growth medium to distilled water that represent the most dramatic change in the salinity. We observed no effect on the OD of the suspension during 250 min after the transfer. Later, the OD began to decrease. During the experiment, all measurements for a sample were completed within approximately 30–60 min following the addition of the concentrated bacterial stock. We are thus confident that the effects of changes in the salinity on the OD were negligible.

D. Measurements of the Refractive Index of the Suspension Medium

We determined the refractive index of the suspension medium at $\lambda = 0.589$ μm using an Abbe-type refractometer (Bausch & Lomb) with a 33–45–58 prism. With this instrument, the refractive index can be measured to 0.001, with visual interpolation to approximately 0.0002. We calibrated the refractometer using three different refractive-index liquids (Cargille Inc.). Three measurements were made for each sample and the results were averaged. For each refractive-index preparation we verified that the refractive index of the sample with bacteria was equal to that of the corresponding reference solution with no bacteria. This assured that our OD measurements with a double-beam spectrophotometer represent solely the effect of beam attenuation by the bacteria and not a residual absorption due to possible slight differences in the concentration of BSA in the sample and reference.

E. Measurements of Optical Density

We measured the OD of cell suspensions with a modified double-beam spectrophotometer (Uvikon 860, Kontron Instruments, Switzerland). In this instrument, the acceptance angle of the detector was limited to less than 1°. Based on the experimental scattering function for bacteria of comparable size[24] we estimate that the error caused by accepting the forward scattered light should be less than approximately −5%. Comparison of our system with a marine transmissometer (Sea Tech, Inc.) with use of suspensions of milk and magnesium oxide powder showed that both instruments yield transmission values that agree to within 5%.

We used standard quartz cuvettes with the optical path of 1 cm for both the sample with bacteria and the corresponding reference solution. The OD measurements (Table 2) were made at a single wavelength of 0.589 μm to be consistent with the determinations of the refractive index of the samples and references. Each OD value was an average of ten measurements in quick succession. The measurements were made with a spectral bandwidth of 2 nm. The instrumental error of the OD measurements is approximately 0.002 at the OD of 1.

Table 2. Measured OD (at a wavelength of 0.589 μm in vacuum) of a Suspension of Free-Living Marine Bacteria as a Function of the Refractive Index n_s of the Immersion Medium

n_s	OD/cm
1.3325	0.20350
1.3431	0.12525
1.3533	0.06325
1.3648	0.02575
1.3704	0.01475
1.3757	0.00738
1.3868	0.00150
1.3920	0.00075
1.4013	0.00738
1.4100	0.02075

It took approximately 8 h to complete all measurements for ten samples. Therefore, to test whether significant changes occurred in the bacterial stock suspension during this time, we also obtained spectra of the OD at the beginning and at the end of the measurement session. The spectra were obtained for the bacterial concentrate diluted 16 times with deionized water. This dilution is equivalent to that of the base sample of the series of ten samples used in the experiment. The spectra were measured between 0.350 and 0.750 μm at 0.001-μm intervals, with a bandwidth of 0.002 μm. From these measurements we found that the OD at $\lambda = 0.589$ μm decreased by approximately 7% during the 8-h measurement session. The slopes s of the OD spectra are essentially the same (1.66 at the beginning and 1.61 at the end of the experiment) if these spectra are fitted to the power-law approximation:

$$\mathrm{OD} = \mathrm{const}\ \lambda^{-s}. \tag{45}$$

This approximation gave an excellent fit: $r^2 = 0.999$ for log OD versus log λ. The small change in the slope s suggests that neither the size distribution of the bacteria nor their refractive index changed significantly during the experiment.

F. Results

The results of OD measurements performed on suspensions of bacteria are shown in Fig. 7 and Table 2. We observed a dramatic reduction in the OD at $\lambda = 589$ nm from 0.204 for $n_s = 1.332$ to approximately 0.001 (essentially the OD measurement error) for $n_s = 1.392$. This reduction is much higher than that previously reported.[10,13] Thus we achieved essentially a full clearing of the bacterial suspension, a striking visual effect. Such clearing can be used for accurate measurements of the absorption of light in bacterial cells[14,25] because the effect of light scattering is essentially nullified.

The minimum OD of the bacterial suspension is observed in our experimental data at a refractive index of the medium equal to 1.3886 at $\lambda = 0.589$ μm. Thus the mean refractive index of the cells $\langle n \rangle$ is

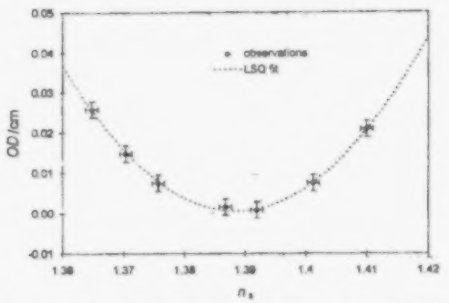

Fig. 7. Optical density OD of a suspension of marine bacteria as measured in solutions of bovine serum albumin with various refractive indices n_s. Only seven data points, roughly centered about the OD_{min}, are shown. The horizontal error bars represent a ± 0.001 n_s measurement error. The vertical bars represent a ± 0.002 OD measurement error. See Table 2 for the OD values, Table 3 for retrieved characteristics of the bacteria, and Fig. 6 for the single-cell size distribution of bacteria.

1.3886 (Table 3). Unfortunately, as mentioned above, without multiwavelength measurements of the OD it was not possible to separate the effects of $\langle k \rangle$ and σ_n^2 on the OD. We nevertheless give estimates of these two latter parameters (Table 3) assuming that the variance of the real part of the refractive index of the particles vanishes (for the estimate of $\langle k \rangle$) or that the particles do not absorb light (for the estimate of σ_n^2). As seen, both $\langle k \rangle$ and σ_n^2 in our experiment are negligible (at an accuracy of 2 standard deviations).

The bimodal size distribution of our bacteria prevented us from validation of our model in terms of $\langle D^4 \rangle^{1/4}$. The $\langle D^4 \rangle^{1/4} \approx 1.15$ μm (Table 3), as determined from the LSQ fit to the OD data (Table 2), is greater than the value of 0.55 μm calculated from the log-normal approximation to the experimental size distribution of single cells (Fig. 6). This is consistent with a significant contribution of the cell aggregates to OD. According to the simulation, approximately 50% of the OD is contributed by particles with $D >$ 1.7 μm (Fig. 1). The significant contribution of the aggregates is also indicated by the small exponential slope (approximately 1.6) of OD as a function of light wavelength [Eq. (45)]. Typically, bacterial suspensions with the average cell size of approximately 0.5 μm and with negligible cell aggregation yield a slope of approximately 2 (Ref. 9).

Table 3. Summary of a Photometric Immersion Refractometry Experiment at a Wavelength of 0.589 μm (in vacuum) with Heterotrophic Marine Bacteria in Bovine Serum Solutions

Parameter and Dimension	Value ± 1 Standard Deviation
$\langle n \rangle$	1.38859 ± 0.00016
$\langle k \rangle$	6.63E-05 ± 4.54E-05
σ_n^2	1.13E-05 ± 7.83E-06
$\langle D^4 \rangle^{1/4}$ (μm)	1.14936 ± 0.00713

Notes: The number concentration of single cells equals 1.3×10^8 cm^{-3}. The $\langle k \rangle$ was calculated with $\sigma_D = 0.4$ (of the experimental single-cell size distribution). The $\langle D^4 \rangle^{1/4}$ was calculated assuming the shape factor $C_s = 1/2$.

6. Conclusions

We have developed and analyzed a rigorous model of the photometric immersion refractometry, a technique to characterize particles in suspensions. This technique is based on a reduction in the beam attenuation of light by a suspension of particles when the real part of the refractive index of the immersion medium approaches the real part of the refractive index of the particles. Such a technique can serve as a benchmark for the refractive-index determination because it does not rely on assumptions inherent in other techniques, such as those based on the inversion of the spectral attenuation data.

We have shown in the numerical simulation that measures of the inhomogeneity of a particle population, both in regard to the refractive index and size as well as to the absorption of light by the particles, can be estimated conveniently with this technique. To demonstrate the applicability of the model, we have carried out a photometric immersion refractometry experiment with heterotrophic marine bacteria.

We successfully clarified the bacterial suspension using bovine serum albumin solution as an immersion medium, and we determined the average real part of the refractive index of cells. The variance of the real part of the refractive index and the imaginary part of the index of cells were found to be negligible in this experiment.

Appendix A: Refractive Index Versus Particle Size

In the model of photometric immersion refractometry we assumed that the refractive index of the particle is independent of the particle size. This assumption is based on microscopic observations with bacteria, which also typically exhibit a low variability of the refractive index.[3] However, it has also been postulated[3,26] that the refractive index may depend on the particle size. Here we examine how the predictions of the model would change in the case of such dependency. We limit our discussion to the case of particles that do not absorb light, thus n is real. However, this discussion can be extended easily to include light-absorbing particles.

From Eqs. (1), (6), (7), and (13) (with k set to 0) we have

$$\mathrm{OD} \approx C_s(\pi^3/\lambda^2 L \log e) \int D^4(n - n_s)^2 F_{DM}(D, n)\mathrm{d}D\,\mathrm{d}n,$$

$$\approx C_s(\pi^3/\lambda^2 L \log e) \int (D^4 n^2 - 2D^4 n\, n_s + D^4 n_s^2) F_{DM}(D, n)\mathrm{d}D\,\mathrm{d}n. \quad \text{(A1)}$$

Equation (A1) can be further transformed as follows, with Eqs. (2), (15), and (16):

$$\mathrm{OD} \approx C_e AN\langle D^4 n^2\rangle - 2C_e AN\langle D^4 n\rangle n_s + C_e AN\langle D^4\rangle n_s^2. \tag{A2}$$

Thus the OD can be approximated with a second-degree polynomial [Eq. (22)]. The coefficients of this polynomial, which can be obtained by one fitting experimental data of the OD versus n_s, can be expressed as functions of the characteristics of the particles as follows:

$$a_0 = C_e AN\langle D^4 n^2\rangle, \tag{A3}$$

$$a_1 = -2C_e AN\langle D^4 n\rangle, \tag{A4}$$

$$a_2 = C_e AN\langle D^4\rangle. \tag{A5}$$

A minimum of the OD occurs at $n_{s\,\min}$ expressed as follows:

$$\begin{aligned} n_{s\,\min} &= -a_1/(2a_2), \\ &= -(-2C_e AN\langle D^2 n\rangle/(2C_e AN\langle D^4\rangle), \\ &= \langle D^4 n\rangle/\langle D^4\rangle. \end{aligned} \tag{A6}$$

We have $n_{s\,\min} = \langle n\rangle$ only if the distribution functions of D and n are independent, i.e., if $F(D, n) = g(D)h(n)$, for in that case $\langle D^4 n\rangle = \langle D^4\rangle\langle n\rangle$. Of course, the OD versus n_s could still be fitted with a parabola if D and n are correlated. However, if the coefficients of the parabola a_0, a_1, and a_2 are used in that case to calculate $n_{s\,\min}$ [Eq. (A6)], the obtained value may poorly represent the average index of the particles $\langle n\rangle$. Similar arguments can be given for other particle characteristics discussed in the context of the immersion refractometry model.

Appendix B: $\langle D^4\rangle^{1/4}$ as an Estimate of $\langle D\rangle$ for a Log-Normal Size Distribution

The $\langle D^4\rangle^{1/4}$ is a biased estimate of the average particle diameter in a population. The magnitude of the bias can be obtained easily, for example, for the particle size distribution expressed with a 0th order log-normal function that was found to adequately describe size distributions of marine particles[27]:

$$g(D) = FD_{\max} \exp[-(\ln D - \ln D_{\mathrm{peak}})^2/(2\sigma_D{}^2)], \tag{B1}$$

where $FD_{\max}$ is a scale constant, D_{peak} is the geometric mean diameter, and σ_D is the width parameter of the distribution. If there are N particles per unit volume of the suspension, then $D_{\mathrm{peak}} = (D_1\, D_2 \ldots D_N)^{1/N}$. For the log-normal distribution [Eq. (B1)], $\langle D^4\rangle^{1/4}$ can be expressed as follows:

$$\langle D^4\rangle^{1/4} = \langle D\rangle \exp[(3/2)\sigma_D{}^2]. \tag{B2}$$

Thus, for a narrow log-normal size distribution ($\sigma_D < 0.1$), we have $\langle D^4\rangle^{1/4} \approx \langle D\rangle$. Most values of σ_D for log-normal components of marine size distributions[27] are within a range of 0.05 to approximately 1, with the most probable value of σ_D at approximately 0.5. Thus, from Eq. (B2), one can expect $\langle D^4\rangle^{1/4}$ to range from $\langle D\rangle$ to $\sim 4.5\,\langle D\rangle$, with the most probable value at approximately $1.5\,\langle D\rangle$.

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TITLE:(U) PHOTOMETRIC IMMERSION REFRACTOMETRY: A METHOD FOR DETERMINING THE REFRACTIVE INDEX OF MARINE MICROBIAL PARTICLES FROM BEAM ATTENUATION

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ABSTRACT: (U) Photometric immersion refractometry is a technique for determining the refractive index of particulate material. In this technique, the attenuation of light by a suspension of particles is measured as a function of the refractive index of the immersion medium. A minimum attenuation occurs at the refractive index of the medium equal to that of the particles. This technique can serve as a benchmark method for the refractive index determination because it is independent of assumptions invoked by other techniques, such as those based on the inversion of spectral attenuation data. We present a rigorous model of the photometric immersion refractometry based on the anomalous diffraction approximation for the attenuation efficiency of particles. This model permits one to determine the average value of the real part of the refractive index of the particles, its variance, and the average imaginary part of the refractive index of the particles. In addition, the fourth moment of the particle size distribution can be determined if the concentration and shape of the particles are known. We analyze the sensitivity of this model to experimental errors and discuss the applicability of photometric immersion refractometry to marine microbial particles. We also present experimental results of this technique as applied to heterotrophic marine bacteria. TRUNCATED

DESCRIPTORS: (U) Mathematical models;Refractivity;Light scattering;Sea water;Backscattering;Particles;Particle size;Photometers;Optical properties;Cells (biology);Aquatic microbiology

IDENTIFIERS: (U) Beam attenuation;Photometric Immersion Refractometry

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